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REVIEW ARTICLE

Subsets in Systemic Lupus Erythematosus

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In recent years, evidence from several laboratories has indicated that various subsets of systemic lupus erythematosus exist. Some of these subsets have distinctive mucocutaneous features but more importantly they have different prevalence of renal disease—thus different prognosis. These subsets are defined by the specificity of the serum antibodies the SLE patient possesses.

Cutaneous lesions are commonly seen in systemic lupus erythematosus (SLE). Approximately 20% of SLE patients will demonstrate prominent cutaneous features at the onset of their disease. Furthermore, 70–80% of SLE patients will, during the course of their disease, display cutaneous features. This data indicates that a dermatologist may play a significant role in the early diagnosis and management of SLE.

SLE is a very heterogenous disease. Some SLE patients have a life-threatening process while others, although displaying many systemic features, have a more benign course. The major course of mortality during the first 5 yr of SLE is renal involvement.

SLE patients make serum antibodies against a variety of cellular macromolecules. These include antibodies against DNA, nuclear protein, histones and various RNA proteins. In addition, SLE patients frequently produce antibodies against nuclear nonnucleic acids and other nuclear and cytoplasmic macromolecules.

During the past 10 yr, clinical investigators have determined that certain antibody systems found in SLE frequently occur in the presence of renal disease, whereas other antibody systems do not. These data indicate that SLE patients can be classified into specific categories (subsets) based upon serum antibodies. This paper is designed to briefly review the clinically significant antibody systems found in SLE and to correlate these antibody systems with various clinical features. To do this, I will rely heavily upon published data and the personal published and unpublished experience obtained together with Drs. Peter J. Maddison and Morris Reichlin in the management of a large lupus clinic.

The chemical nature of the antigen, the fluorescent staining pattern and the methods of detection of these clinically important antibody systems are presented in Table I. The designation of these various antibody systems is an abbreviation of the surname of the patient in whose serum the antibody system was originally detected. The clinical features associated with the specific antibodies are presented in Table II.

I. Anti-Native DNA (nDNA) Antibodies [1,2]

Ample evidence now exists indicating that the presence of anti-nDNA antibodies almost always indicates a severe prognosis. These patients have a high incidence of clinical renal disease and hypocomplementemia. Elution studies from kidneys of patients dying with SLE have demonstrated an enhanced concentration of anti-DNA antibodies suggesting a cause and effect relationship between the presence of DNA, anti-DNA immune complexes and the renal pathology. At times, anti-nDNA antibodies and hypocomplementemia may antedate the appearance of clinical renal disease but kidney biopsies of such patients will almost always detect a significant immunological insult. The lupus band test (LBT), employing noninvolved upper forearm skin, is positive 80–90% of the time in patients possessing anti-DNA antibodies [3,4]. More important, the LBT is usually composed of IgG alone or IgG together with other immunoglobulin classes [4]. Following successful therapy with immunosuppressive agents and/or oral steroids, the anti-nDNA antibodies disappear, the LBT becomes negative and serum complement levels return to normal. Reexacerbation of the lupus process is frequently accompanied by a reappearance of the anti-nDNA, hypocomplementemia and a positive LBT.

Anti-nDNA antibodies are most commonly measured by 1 of 3 techniques. These are: counterimmunoelectrophoresis, radioimmunoassay and most recently an immunofluorescent technique employing Crithidia luciliae as a substrate [5].

The techniques of counterimmunoelectrophoresis and radioimmunoassay can be found in any standard immunologic textbook. Crithidia luciliae is a nonpathogenic hemoflagellate containing a large mitochondrium composed of circular DNA (nDNA). Test sera of various dilutions are layered over the organisms, incubated, washed and then stained with a fluorescein conjugated anti human globulin antiserum. A positive test is depicted in Fig 1.

Recent preliminary evidence in our laboratories suggests complement fixing anti-single stranded (ss) DNA antibodies may also be pathogenetically significant in the production of lupus nephritis. We have detected several SLE patients with severe nephritis observed over a period of years who have repeatedly failed to demonstrate anti-nDNA antibodies (by Crithidia assay) but who have possessed serum complement fixing anti ssDNA antibodies.

II. Antinuclear Ribonucleic Acid Protein (anti-nRNP) (Mo) [6,7]

This antibody system is directed against heat labile nuclear RNP and has been described in patients demonstrating features of the mixed connective tissue syndrome, scleroderma and SLE. Sharp et al [6] were the first to describe under the heading of the mixed connective tissue syndrome, a group of patients possessing anti-nRNP antibodies and having clinical features of

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Abbreviations:
ARA: American Rheumatism Association
CTE: calf thymus extract
ENA: extractable nuclear antigen
LBT: lupus band test
SLE: systemic lupus erythematosus

TABLE I. *The chemical nature of the antigen, the fluorescent staining pattern, and the methods of detection of important antibody systems*

Antigen	Chemical character	Antinuclear antibody fluorescent pattern (mouse liver substrate)	Method of detection
nDNA	DNA-protein	Rim or peripheral Pattern	Counterimmunoelectrophoresis; radioimmunoassay; Crithidia luciliae
nRNP (Mo)[a]	RNA-protein	Speckled (high titered)	Hemagglutination Gel double diffusion
Sm[a]	Nuclear glycoprotein	Speckled	Hemagglutination Gel double diffusion
SSB[b] (Ha)[a]	Nuclear glycoprotein	Speckled	Gel double diffusion
Ro[a]	Cytoplasmic glycoprotein	None	Gel double diffusion
La[a]	Cytoplasmic RNA protein	None	Gel double diffusion

[a] The designation of these antibody systems represent an abbreviation of the patients' name in whose serum the antibody was initially detected.
[b] Designation represents B antigen of Sjogren's syndrome.

TABLE II. *Clinical features associated with specific antibodies in SLE*

Antibody	Clinical features
DNA	Renal disease
nRNP	Incidence of renal disease mixed connective tissue disease, polyserositis, myositis and Raynaud's phenomena
Sm	Renal disease
SSB (Ha)	Sicca syndrome
La	Sicca syndrome
Ro	Photosensitive dermatitis Renal disease Sicca syndrome

both SLE and scleroderma. Their 25 patients demonstrated many features of scleroderma (e.g., swollen hands, Raynaud's phenomena, abnormal esophageal motility) and indeed 4 of their patients displayed widespread sclerodermatous changes. Further, most of their patients responded to treatment with immunosuppressive agents and/or steroids.

Reichlin and Mattioli [7] have demonstrated that approximately 33% of patients satisfying the American Rheumatism Association's (ARA) preliminary criteria for SLE, possess anti-nRNP antibodies. In a comparative study between SLE patients possessing only anti-DNA antibodies and those possessing only anti-nRNP, these authors failed to note the sclerodermatous features found by Sharp et al in their patient population. Their anti-nRNP positive patient population, on the contrary, was similar to the DNA positive SLE group in all respects except for the increased prevalence of pleuropericardial serositis, myositis and Raynaud's phenomenon in the nRNP group. All studies of SLE patients demonstrating only anti-nRNP agree, however, that anti-nRNP antibodies are found in an SLE and mixed connective tissue patient population with a low prevalence of renal disease [6,7]. This latter point is most important to emphasize for, in our observation of over 100 SLE patients, we have been unable to clinically distinguish the majority of these patients possessing anti-nRNP antibodies from those who do not. The clinical experience of our group indicates that the patient population possessing only anti-nRNP is very heterogenous. This experience is summarized in Table III [8]. As one can see, at one extreme are a relatively small group (perhaps 10–15%) of anti-nRNP positive patients with clinically obvious mixed connective tissue features of SLE and scleroderma as described by Sharp et al. At the other extreme are anti-nRNP positive patients with constitutional but not cutaneous signs and symptoms of a connective tissue disease. In the middle, constituting the largest population of nRNP positive patients are a group of lupus patients, the vast majority satisfying the ARA criteria for the diagnosis of SLE. The major point to reemphasize is that SLE patients possessing only anti-nRNP antibodies have a very low incidence of renal disease, hence they have an excellent prognosis.

The anti-nRNP antibodies are detected by 2 methods: a passive hemagglutination assay using saline soluble extractable nuclear antigens (hence the term extractable nuclear antigen [ENA], employed by Sharp, in context with the mixed connective tissue) adsorbed onto sheep red blood cells [6]. These cells then are reacted with various dilutions of test serum. Hemagglutination of the red blood cells indicate the presence of serum antibody directed against antigens absorbed onto the red blood cells.

The second technique employs gel double diffusion and calf thymic extract (CTE) as a source of nuclear antigen. The CTE

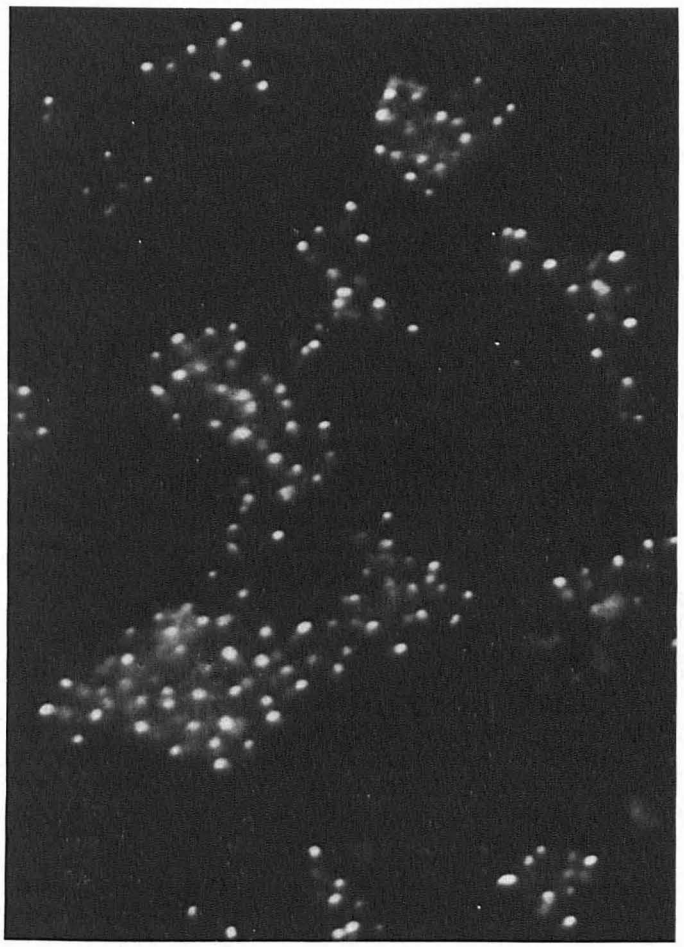

FIG 1. Positive Crithidia luciliae assay for anti-nDNA antibodies. Mitochondria containing (nDNA) stain with fluorescent conjugated anti-human globulin serum. Serum dilution 1:20 from an SLE patient with renal disease and hypocomplementemia (reduced from × 400).

TABLE III. *Clinical diagnoses in 43 patients demonstrating only anti-nRNP antibodies [8]*

Diagnosis	Number of Patients
Mixed connective tissue disease	5 (12%)
Progressive systemic sclerosis	1 (2%)
Systemic lupus erythematosus	34 (80%)[a]
Rheumatoid arthritis	1 (2%)
Drug induced SLE	1 (2%)
Non-Rheumatic disease	1 (2%)

[a] 30 (71%) satisfy preliminary criteria of American Rheumatological Association for SLE.

is placed in the center well and reacted with the test serum. A precipitin line indicates the presence of serum antibodies against an antigen present in the CTE. The specificity of the reaction is determined employing reference serum known to contain anti-nRNP. Figure 2 is a schematic representation of this technique.

SLE patients containing anti-nRNP are frequently hypergammaglobulinemic. Surprisingly, one study indicates that as much as 20% of the gammaglobulin fraction of these patients may contain antibody directed against nRNP. This could be very significant since nRNP-anti nRNP immune complexes would tend to form in antibody excess. These complexes would be insoluble and thus be readily cleared by the reticuloendothelial system. Thus, the relative ease of clearance of the nRNP-anti nRNP immune complexes by the reticuloendothelial system could conceivably explain the low incidence of renal disease in those SLE patients possessing only anti-nRNP antibodies. By contrast, SLE patients make relatively small amounts of anti nDNA antibodies. Thus, nDNA-anti nDNA immune complexes tend to form in antigen excess and are soluble. These complexes escape clearance by the reticuloendothelial system and, thus, conceivably are able to take part in the immune complex deposition in the kidney.

The LBT in those patients possessing only anti-nRNP is usually either negative or contains only IgM [4]. *In vivo* speckled epidermal ANA's have been noted by several groups in biopsies of normal skin of these patients. These patients all contained high titer anti-nRNP.

III. Anti-Smith (Anti-Sm) Antibodies [9,10]

Antibodies against the acidic nonnucleic acid nuclear macromolecule termed Sm are found in approximately 25% of SLE patients. This antibody system appears to be highly specific for SLE and preliminary evidence indicates that lupus patients having only this antibody system are a subset of SLE patients prone to develop renal disease [10].

This antibody system is measured by passive hemagglutination techniques as described above. The RNP portion of the extractable antigen on the sheep red blood cells is destroyed by RNAase prior to performance of the hemagglutination assay for Sm. The Sm system can also be detected employing gel double diffusion and standard reference serum.

IV. Anti SSB [11] and Ha [12] Antibodies

These 2 antibody systems described by 2 different groups appear, by gel double diffusion, to be identical antibody systems. In this paper, these antibody systems will be referred to as SSB. Thus far, the preliminary reports suggest this antibody system reactive against a soluble nonnucleic acid labile acid nuclear protein antigen, giving a speckled nuclear fluorescence, is found in approximately 15% of SLE and 30% of Sjogren's syndrome patients. Furthermore, those SLE patients possessing these antigens appear to have an increased prevalence of Sjogren's syndrome and rheumatoid factor. The rheumatoid factor present in the serum is not related to the anti-SSB antibodies.

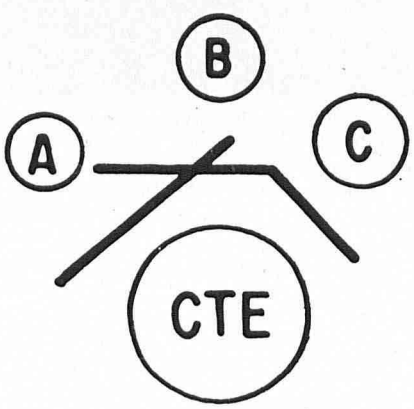

FIG 2. Test serum B demonstrating precipitin line against calf thymic extract (*CTE*) and a line of identity with known antibody system in reference serum placed in *well C*. The test serum *B* demonstrates a line of nonidentity with known antibody system present in reference serum *A*.

V. Anti La Antibodies [13]

The La antigen is a RNA cytoplasmic protein macromolecule. The anti-La antibody is found in 10–15% of SLE patients and approximately 30% of Sjogren's patients. Those SLE patients possessing this antibody system tend to have a high incidence of the sicca (Sjogren's) syndrome.

VI. Anti-Ro Antibodies [5,14]

Antibodies against this cytoplasmic, acidic nonnucleic acid glycoprotein macromolecule of approximately 100,000-150,000 mw appear to be very specific for SLE and Sjogren's syndrome. Approximately 25% of SLE patients possess this antibody in their serum as do 30% of Sjogren's patients. Of 72 Ro positive (+) patients, 58 (81%) displayed features compatible with the diagnosis of SLE, 7 (10%) possessed a nonlupus collagen vascular disease and 6 (8%) had no evidence of a collagen vascular disease. These latter 6 Ro+ patients were detected in a screening of over 5,000 hospitalized patients' sera. Those SLE patients demonstrating the Ro antibody system appeared to have an increased prevalence of photosensitivity, renal disease, Sjogren's syndrome and rheumatoid factor [14]. Approximately 75% of Ro patients also possess anti La antibodies.

A direct role for antigen-antibody complex formation involving anti-Ro antibodies in the pathogenesis of the renal disease is suggested by elution studies of several SLE patients dying of renal disease which demonstrated an enhanced anti-Ro antibody concentration in the kidneys.

Further studies have detected a group of Ro+ lupus patients who fail to demonstrate antinuclear antibody by routine immunofluorescent techniques employing mouse liver as a nuclear substrate. These patients may demonstrate a positive ANA when human tissue is utilized as a nuclear substrate (e.g. human spleen). These patients frequently satisfy the ARA criteria for the diagnosis of SLE. A subset of these "ANA negative" patients display a prominent photosensitive dermatitis which, in our experience, has demanded antimalarials and/or oral corticosteroids to control. Our preliminary findings suggest that a large segment, perhaps as many as 50% of "ANA negative" lupus patients displaying an intense photosensitive widespread dermatitis possess this antibody system [15]. There is no direct relationship between these antibodies and the widespread dermatitis since 50% of patients with a similar if not identical widespread photosensitive dermatitis fail to demonstrate anti Ro. The point to be stressed is that these "ANA negative" patients possess a precipitin antibody system commonly found in SLE patients.

The LBT in Ro+ patients is generally negative and if positive usually demonstrates only IgM deposition [4].

The anti-Ro antibodies are demonstrated by gel double diffusion utilizing human spleen or liver saline extracts as a source of antigen.

It must be emphasized that many SLE patients form antibodies against several of these antigens. Thus, anti-nDNA antibodies may be seen with anti-Sm antibodies but infrequently with anti-nRNP. Likewise, anti-SM and anti-RNP appear frequently in the same patient. In these circumstances the prognosis appears to be determined by the antibody associated with the worse prognosis. Thus, SLE patients possessing anti-Sm and anti-RNP antibodies appear to have an incidence of renal disease similar to those patients possessing anti-Sm alone. In a similar fashion the presence of anti-DNA antibodies irrespective of the types of additional serum antibodies appears almost always to carry a potentially serious prognosis.

This brief discussion has attempted to summarize the state of the "art" of serological investigations of SLE patients and their correlation with clinical features. These tests performed upon newly recognized SLE patients have given us the ability to detect those SLE patients at risk to develop renal disease. Thus these tests offer the practicing physician a valuable prognostic indicator in the evaluation of SLE patients.

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Announcement

The 40th Annual Meeting of the Society of Investigative Dermatology will be held on May 7–9, 1979 at the Sheraton-Park Hotel, Washington, DC.

Announcement
National Psoriasis Foundation
Summer Scholarships for Medical Students

Predoctoral summer fellowships will be available for support of psoriasis-related research. Maximum stipend is $1,000. Deadline for applications is April 15, 1979. Applications may be obtained from: Sheldon R. Pinnell, M.D., Professor of Medicine, Box 3135 Hospital, Duke University Medical Center, Durham, North Carolina 27710.